OLIGONUCLEOTIDE DENDRIMERS: FROM POLY-LABELLED DNA PROBES TO STABLE NANO-STRUCTURES

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Introduction

Dendrimers are discrete, highly branched, monodispersed polymers that possess patterns reminiscent of the branching of trees. Dendrimer oligonucleotides are representative of a new segment of polymer science[1].

Two strategies for the synthesis of oligonucleotide dendrimers are possible: Divergent - with the structure growing from the center to the periphery; and Convergent - with growth from the periphery to the center. The number of branches generated at each step determines the number of repetitive steps necessary to build up the desired molecule and the density of groups at the periphery. The groups or moieties on its outer shell determine the properties of the molecule.

Applications proposed for dendrimers exploit the high density and the large number of groups on the periphery. For example, dendrimers with a positively charged surface interact strongly with nucleic acids, helping to transport them through the membranes of cells[2]; dendrimers with internal cavities interact non-covalently with guest molecules to give guest-host systems[3]. In oligonucleotide chemistry, branched oligonucleotides[4] were used for signal amplification in hybridization tests, making it possible to detect the target at a level of below 100 molecules/mL[5].

Synthesis of Oligonucleotide Dendrimers

Described here is a set of branching phosphoramidite synthons which can expand the potential of oligonucleotide synthesis, opening ways to 2D and 3D structures. The set, shown in Scheme 1, consists of symmetric and asymmetric doublers (1) and (2)[6] and a symmetric trebler (3)[7]. These phosphoramidite products permit the synthesis of a dendrimeric structure on top of a conventional "monomeric" oligonucleotide, as well as directly on the solid support. The monomeric and the dendrimeric sequence segments can be prepared with different lengths and different orientation by using 3'- and 5'-nucleoside phosphoramidites. The branches can terminate in any moiety available from the arsenal of phosphoramidite synthons. In this way using current synthesis technology, the symmetric doubler and trebler can be easily exploited to develop molecules like the symmetrical examples shown in Scheme 2, with a variety of desirable features.

Previously, an asymmetric branching reagent[6] has been used to prepare comb structures which offer good control of the number and spacing of hybridization sites. Novel applications for dendrimers could also be opened up if mixed terminal functionalities (*e.g.*, oligonucleotides, reporter groups) were introduced onto the outer surface of dendrimers to give asymmetric structures like the example shown in the lower right of Scheme 2. These can be introduced at any generation of dendrimer synthesis using the asymmetric doubler (2), which contains two primary hydroxy groups protected with Fmoc and DMT. If different chemistries are

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Probe Purification

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Novel Monomers

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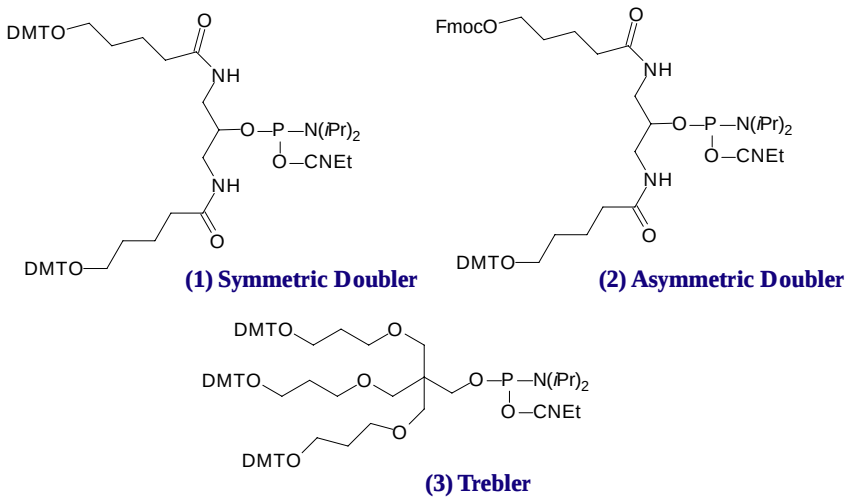
then applied along the two different branches opened by different protecting groups, the result will be a dendrimer with two different functionalities on the surface and (possibly) different internal links. The generation at which the asymmetric doubler is introduced will determine the relative contribution of the two functionalities. After addition of the asymmetric doubler, its DMT-protected arm is first used to build up a desired structure. After final detritylation and capping the Fmoc group is deprotected[8] and the synthesis is then carried out to generate the second half of the structure, with subsequent final deprotection.

When using 5-10 min condensation time, the doublers (1) and (2) give high condensation yields (>95%) for up to 4 condensations. The trebler (3) gives stable trebling for up to 3 condensations when using 500Å CPG supports, and 4-5 condensations using a 2500Å CPG support, thus giving *ca* 80-240 terminal hydroxyl groups. Elongation of branches using propan-1,3-diol or ethyleneglycol-based linkers further improves the yield of the next condensation with the trebler, as does adding the spacer between the first trebler and the solid support.

Spacer phosphoramidites added directly after the branching phosphoramidites can be used to reduce the density of packing of the dendrimer branches[7]. Spacers can also affect the size, flexibility, solvation properties and chemical functionality of the dendrimers.

SCHEME 1: SYNTHESIS OF OLIGONUCLEOTIDE DENDRIMERS



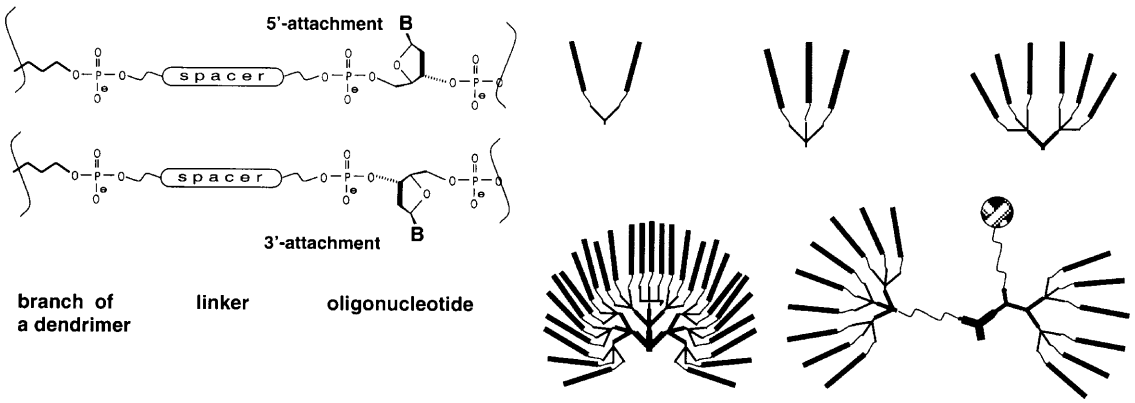
Labelled Oligonucleotide Dendrimers

Dendritic oligonucleotides bearing several biotin residues, fluorophores or radioactive isotopes can be detected at much lower concentrations due to multiple labelling. Such amplification of signal may be particularly important in *in situ* hybridization and in the emerging techniques which exploit oligonucleotide arrays[9, 10], where the signal is limited by the surface density of the device. But too dense a concentration of reporter groups leads to self-quenching of fluorescence. It also makes some oligonucleotide branches unavailable for hybridization and renders them inaccessible to bulky enzymes. In these circumstances the use of spacers is necessary.

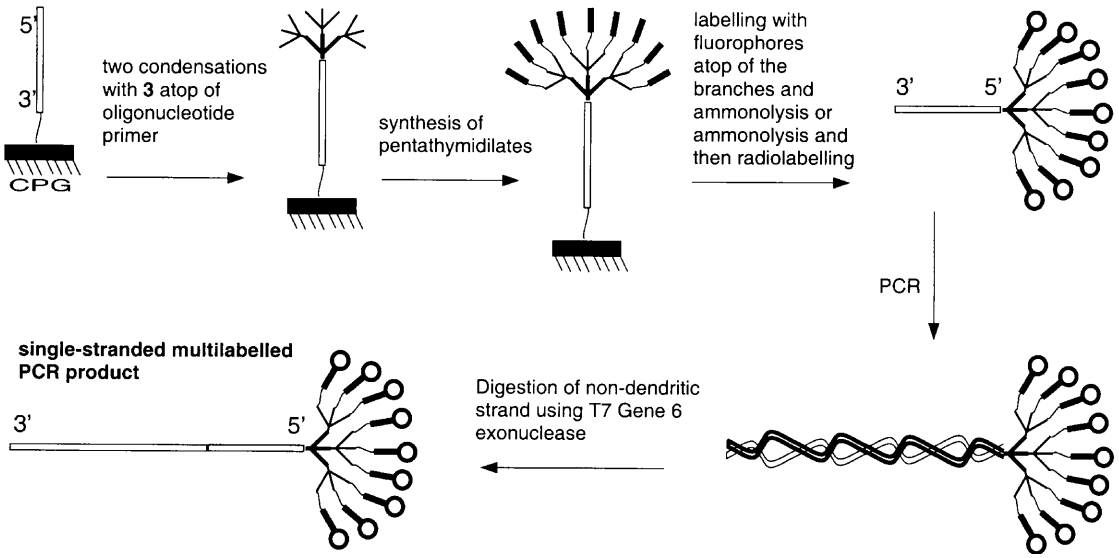
Dendrimers containing 'bouquets' of short oligonucleotides can be labelled with γ-^{32}P-ATP and polynucleotide kinase. When used as probes to oligonucleotide arrays, the multiply-labelled structures show much higher sensitivity than their mono-labelled counterparts. The large dendrimeric structure does not affect the hybridization yield, even with oligonucleotides tethered to a surface[9,10,11,12].

Besides the obvious means of amplifying the signal, it is possible, using synthons with different protective groups, to introduce different labels on the ends of different branches. These could be fluorophores with different emission spectra, providing the potential for a wide palette of colors; or the different ends

SCHEME 2: EXAMPLES OF PLAIN AND MIXED DENDRIMERS OF DIFFERENT GENERATIONS



SCHEME 3: CONVERSION OF A POLYLABELLED PCR PRIMER INTO A SINGLE-STRANDED POLYLABELLED PCR PRODUCT

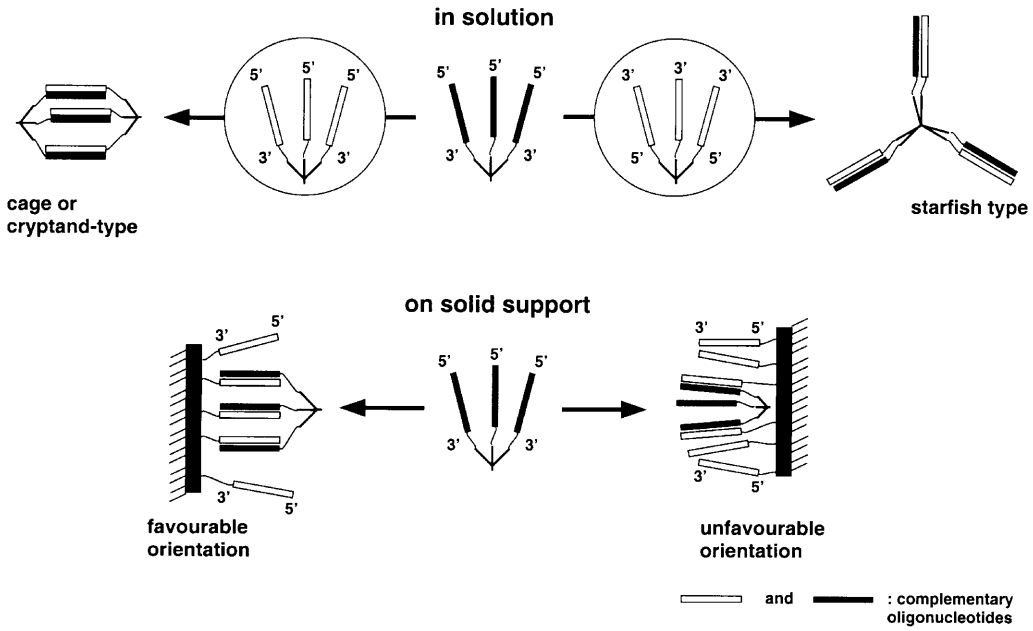


could bear the donor and acceptor of an energy transfer pair; or just a different sequence on each branch.

Oligonucleotide Dendrimers as PCR Primers

A range of applications is opened up when dendrimeric oligonucleotides are incorporated into products by PCR to prepare, for example, probes with higher labelling capacity. Dendritic primers are indeed compatible with PCR conditions. The branched structure has a considerable effect on the electrophoretic mobility in that the PCR products have reduced mobility in gel electrophoresis. Products of PCR are double-stranded and therefore make poor hybridization probes without further treatment. Many of the methods for making probes, such as an asymmetric PCR, are cumbersome. The structure of the dendrimer is such that it leaves a 'bouquet' of oligonucleotides as a 5'-overhang on the double-stranded PCR product. Such a

SCHEME 4: FORMATION OF COMPLEMENTARY DENDRITIC COMPLEXES



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structure is naturally resistant to a number of exonucleases, and double-stranded PCR products are readily converted to single strands by T7 Gene 6 exonuclease[7], as shown in Scheme 3.

Mobility Shift by Dendrimeric Tags

It can be useful to modify the electrophoretic mobility of a PCR product by attaching a polymeric tag to one of the primers. The resulting mobility shift can be used to spread a set of multiplexed PCR products so that they do not interfere with each other. The addition of a dendrimeric 'bouquet' of short oligonucleotides to a PCR primer has a profound effect on the mobility of the PCR product. Addition of 9 pentathymidines decreased the mobility in agarose gels by an amount equivalent to adding 50 base pairs. This effect is even larger in polyacrylamide gels because of the smaller pore size. The number of couplings required to make the branched tag is small, especially when compared with the number of couplings required to make an unbranched polymer with the same effect on mobility. A total of 7 steps are used to make the dendrimeric tag: 50 steps would be needed to add 50 bases.

Nano-structures from DNA Dendrimers

Controllable formation of nano-scale architectures in solution and on solid supports is central to a range of activities in the emerging field of nanotechnology[13]. DNA molecules are well suited for these purposes because of their unique molecular recognition features. For example, dendrimers with arms terminating in oligonucleotides of the same or of different sequences could be used to build cages, cryptands, tubes, nets, scaffolds and other more complex 3-D structures[11], shown in Scheme 4.

A different range of structures is possible when dendrimers interact with oligonucleotides bound to a solid support, which essentially represent a dendrimer with an infinite number of branches and a single core. In this case, the formation of an infinite network is not possible. When all oligonucleotides on the surface are involved in duplex formation, the complements will form a monolayer on the surface. However, growth of structures from the surface can be seeded from dendrimers which have some branches used to tie to the surface and others which are not complementary to the oligonucleotides on the surface. Complex patterns of oligonucleotides with different sequences can readily be synthesized on surfaces, for example, by light directed methods[12] or a physical masking method[9,10].

Duplexes formed from dendritic DNA have unexpectedly high thermal stability; the melting temperature is substantially higher than that of linear counterparts. It is an effect of combining the binding forces of individual duplexes by tying their ends together in the dendritic structure.

Conclusions

- Plain and mixed oligonucleotide dendrimers can be synthesized using novel doubling and trebling phosphoramidite synthons.
- Incorporation of label using γ-^{32}P-ATP and polynucleotide kinase increases in proportion to the number of 5'-ends.
- Fluorescent signal also increases in proportion to the number of 5'-ends, if spacers are incorporated between the labels and the ends of the branches.
- When using a dendrimeric oligonucleotide as a PCR primer, the strand bearing the dendrimer is resistant to degradation by T7 Gene 6 exonuclease making it easy to convert the double-stranded product of the PCR to a multiply labelled, single-stranded probe.
- For DNA dendrimers of different generations reassociated as complementary pairs in solution or with an array of complementary oligonucleotides on a solid support, duplex stability is greater than that of unbranched molecules of equal length. Enhanced stability of DNA dendrimers makes them useful as building blocks for the 'bottom up' approach to nano-assembly.
- These features also suggest applications in DNA chip technology when higher temperatures are required, for example, to melt secondary structure in the target.

References:

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ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Symmetric Doubler Phosphoramidite	10-1920-90	100 µmole	75.00
	10-1920-02	0.25g	240.00
Asymmetric Doubler Phosphoramidite	10-1921-90	100 µmole	90.00
	10-1921-02	0.25g	300.00
Trebler Phosphoramidite	10-1922-90	100 µmole	90.00
	10-1922-02	0.25g	300.00

TOM-PROTECTED MINOR BASE RNA PHOSPHORAMIDITES

RNA synthesis using monomers containing the 2'-O-Triisopropylsilyl-OxyMethyl (TOM) group (TOM-Protecting-Group™) is characterized by very high coupling efficiency along with fast, simple deprotection. High coupling efficiency is achieved because the TOM-Protecting-Group exhibits lower steric hindrance than the 2'-O-t-butyl-dimethylsilyl (TBDMS) group used in our alternative RNA monomers. Fast and reliable deprotection is achieved using methylamine in ethanol/water at room temperature. A further feature of the TOM-Protecting-Group is that during basic steps it can not undergo 2' to 3' migration. This migration under basic conditions leads to non-biologically active 2'-5' linkages when using the TBDMS group. These features allow the TOM-Protected monomers to produce longer oligonucleotides.

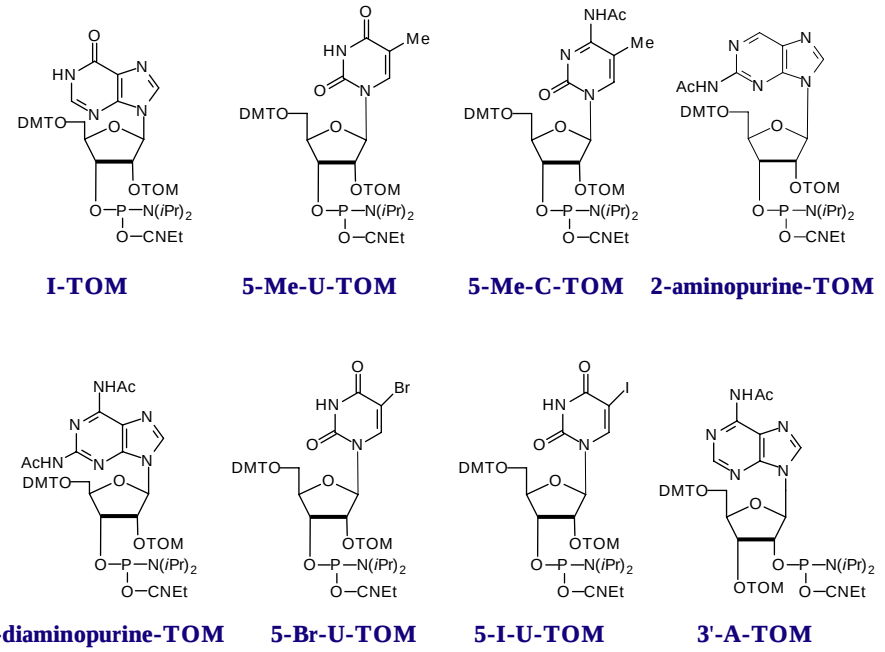
The regular TOM-Protected RNA monomers have achieved considerable acceptance by a growing number of our customers. Now we are beginning to offer minor bases with the TOM-Protecting-Group. Although these are intended for use with the regular TOM-Protected RNA monomers, they are also fully compatible with the use of monomers with 2'-O-TBDMS protection. (See Page 43 of our 1999 Catalog for the regular RNA monomers and supports with the TOM-Protecting-Group.)

Initially, we have prepared the minor bases most requested by our customers and they are shown in Figure 1 and described in very basic terms below. Inosine, 5-Methyl-Uridine, 5-Methyl-Cytidine, 2-Aminopurine riboside and 2-Amino-Adenosine are useful for analyzing RNA structure and activity relationships, for example, in ribozyme studies. 5-Bromo-Uridine and 5-Iodo-Uridine have been used for crystallography studies and cross-linking experiments. 3'-Adenosine is used to prepare oligonucleotides with non-standard 2'-5' linkages.

We will be happy to prepare further minor bases, so please contact us with your favorite structure.

TOM-Protecting-Group is a trademark of Xeragon AG, Zurich and patents are pending.

FIGURE 1: STRUCTURES OF MINOR BASES WITH TOM-PROTECTING-GROUP



ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
I-TOM-CE Phosphoramidite	10-3044-95	50µmole	95.00
	10-3044-90	100µmole	190.00
	10-3044-02	0.25g	475.00
5-Me-U-TOM-CE Phosphoramidite (T)	10-3054-95	50µmole	95.00
	10-3054-90	100µmole	190.00
	10-3054-02	0.25g	475.00
5-Me-C-TOM-CE Phosphoramidite	10-3064-95	50µmole	95.00
	10-3064-90	100µmole	190.00
	10-3064-02	0.25g	475.00
2-Aminopurine-TOM-CE Phosphoramidite	10-3074-95	50µmole	212.50
	10-3074-90	100µmole	425.00
	10-3074-02	0.25g	975.00
2,6-Diaminopurine-TOM-CE Phosphoramidite (2-amino-A)	10-3084-95	50µmole	212.50
	10-3084-90	100µmole	425.00
	10-3084-02	0.25g	975.00
5-Br-U-TOM-CE Phosphoramidite	10-3094-95	50µmole	95.00
	10-3094-90	100µmole	190.00
	10-3094-02	0.25g	475.00
5-I-U-TOM-CE Phosphoramidite	10-3095-95	50µmole	95.00
	10-3095-90	100µmole	190.00
	10-3095-02	0.25g	475.00
3'-A-TOM-CE Phosphoramidite	10-3604-90	100µmole	40.00
	10-3604-02	0.25g	100.00

POLY-PAK PURIFICATION OF LABELLED PROBES

Introduction

The demand for fluorescently labelled oligonucleotides has been steadily increasing. With the advent of fluorophores supplied as both CPG and phosphoramidites, oligo synthesis has become routine and efficient. Yet their purification remains a bottleneck in production, due primarily to the failure sequences being more hydrophobic than the full length oligo when a 3'-dye is present. What is needed is a means by which the oligo can be first purified by length, and then afterwards, by means of the dyes to remove any unlabeled sequences. A new Poly-Pak purification protocol developed at Glen Research allows one to do exactly this.

Protocols are tailored to specific dye combinations such as those seen in FRET or Molecular Beacon probes. The strategy behind the purification lies in washing the Poly-Pak cartridge with acetonitrile solutions at low and neutral pH. After first loading the oligo on to the cartridge, the phosphodiester backbone is protonated using 2% TFA. Then a rinse is performed with an acetonitrile/2% TFA solution (Solution A). With the phosphate backbone protonated, the hydrophobicity of the oligo increases as a function of length and so, the failure sequences are selectively removed without loss of full length product. Then the pH is brought back to neutrality and a second wash with an acetonitrile/TEAA solution (Solution B) is performed. At pH 7, the presence of the dyes is the deciding factor in the oligo's affinity to the Poly-Pak support and any sequences without the dyes present, or intact, are removed.

Table 1 contains a general description of the procedure, while details of the wash Solutions A and B are collected in Table 2. Examples of the purification results are shown in Figures 1-9. Some notes on individual probe types follow:

Molecular Beacons

This protocol has been optimized using molecular beacons of 30 nucleotides in length that contain either fluorescein or Cy5 at the 5' terminus and Dabcyl on the 3' end. Using the Poly-Pak™ II cartridge, 0.5 µmole of crude oligo (approximately 50 OD units) may be purified; overloading the cartridge results in lower probe yield and purity. Due to the difference in hydrophobicity of the Cy5 and fluorescein, different rinsing solutions are used. For beacons that contain fluorophores of intermediate hydrophobicity, *e.g.*, TAMRA, try an intermediate concentration of ACN in the TEAA buffer for Solution B.

FRET Probes

This protocol has been optimized for 16-20mers containing both Fluorescein and TAMRA on a 0.5 µmol scale (approximately 50 OD units);

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TABLE1: POLY-PAK MATERIALS AND PREPARATION STEPS

Materials	***Amount Used***
Poly-Pak™ II Cartridge	1
HPLC grade Acetonitrile (ACN)	4 mL
2.0 M Triethylammonium Acetate (TEAA) pH 7	5 mL
Deionized Water (dH_2O)	12 mL
2% Trifluoroacetic Acid (TFA) in Water (2% TFA)	4 mL
50% Acetonitrile in Water	3-4 mL

Cartridge Preparation

The flow rate of solvents through the Poly-Pak cartridge should be dropwise.

1. Connect a syringe to the female luer of the cartridge and have the male luer terminate in a waste vessel.
2. Flush the cartridge with 4 mL acetonitrile followed by 4 mL 2 M TEAA.

Sample Preparation

3. Following synthesis, deprotect the oligo. If 50 mM K_2CO_3 in anhydrous methanol has been used, continue to the next step. If ammonia or AMA was used for deprotection, dry the probe down, take up in 1 mL dH_2O and then continue with the following step.
4. Add 1 volume 2 M TEAA followed by 8 volumes of deionized water.

Purification Procedure

5. Load the sample solution onto the cartridge . Reload if necessary after addition of a few drops of 2 M TEAA which increases the affinity of the oligo for the support.
6. Flush cartridge with 5 mL 2% TFA; do this whether the oligo is DMT ON or OFF.
7. Rinse cartridge with 15 mL of Solution A.
8. Flush cartridge with 4 mL dH_2O.
9. Rinse cartridge with 10 mL of Solution B.
10. Elute product with 3-4 mL of 50% Acetonitrile in Water.

TABLE 2: WASH SOLUTIONS

Molecular Beacons

Solution A: ACN : 2% TFA (1:1.5)

Solution B: 14% ACN in 0.1 M TEAA *(fluorescein oligos)*
25% ACN in 0.1 M TEAA *(Cy5 oligos)*

FRET Probes

Solution A: ACN : 2% TFA (1:2)

Solution B: 12% ACN in 0.1 M TEAA

3'-Dye-Labelled Oligos

Solution A: ACN : 2% TFA (1:3)

Solution B: 10% ACN in 0.1 M TEAA

(If significant TAMRA or fluorescein is seen coming off cartridge, stop the rinse.)

Notes

The percent ACN used in Solution A rinse depends mostly upon the oligo length, though the type and number of dyes present also is a factor. In terms of length, a rule of thumb is to increase the percent ACN by 0.5% per base added. The percent ACN in Solution B is most strongly dependent upon the hydrophobicity of dye(s) labelled on the oligo and is independent of oligo length. An oligo with two very hydrophobic dyes such as Cy5 and Dabcyl can require up to 25% ACN in 0.1 M TEAA.
Finally, if the oligo does not bind quantitatively to the cartridge during loading, lower the pH by adding a few drops of glacial acetic acid. Conversely, if there is difficulty getting all the purified oligo off the cartridge when eluting, add a drop of 30% ammonia to the 50% ACN.

FIGURE 1: RP HPLC OF CRUDE MOLECULAR BEACON

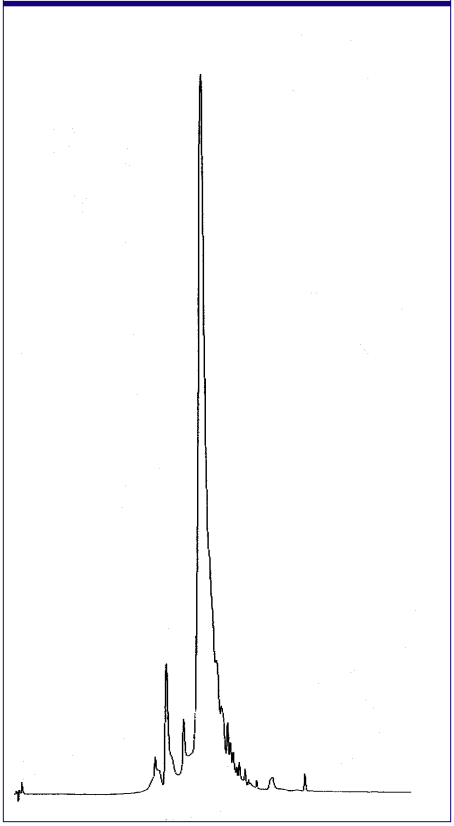


FIGURE 2: RP HPLC OF PURIFIED MOLECULAR BEACON

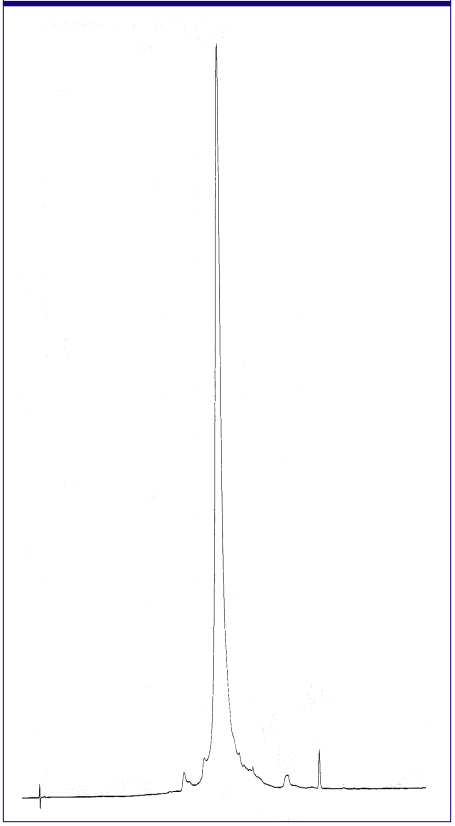


FIGURE 3: UV-VIS SPECTRUM OF PURIFIED MOLECULAR BEACON

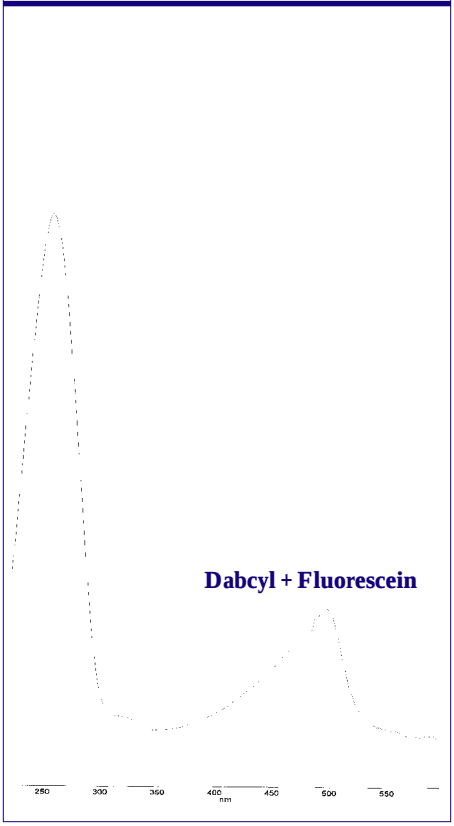


FIGURE 4: RP HPLC OF CRUDE FRET PROBE

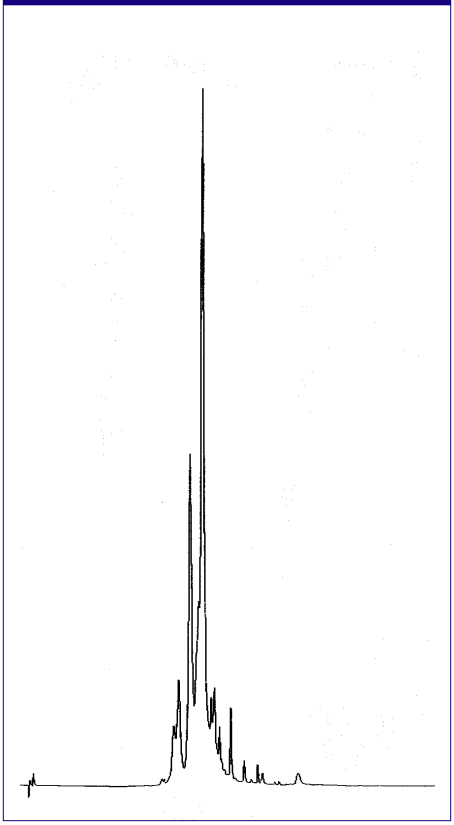


FIGURE 5: RP HPLC OF PURIFIED FRET PROBE

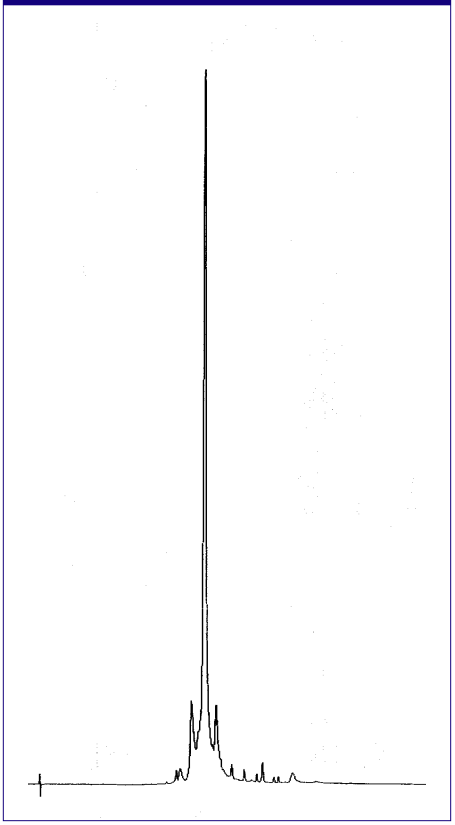


FIGURE 6: UV-VIS SPECTRUM OF PURIFIED FRET PROBE

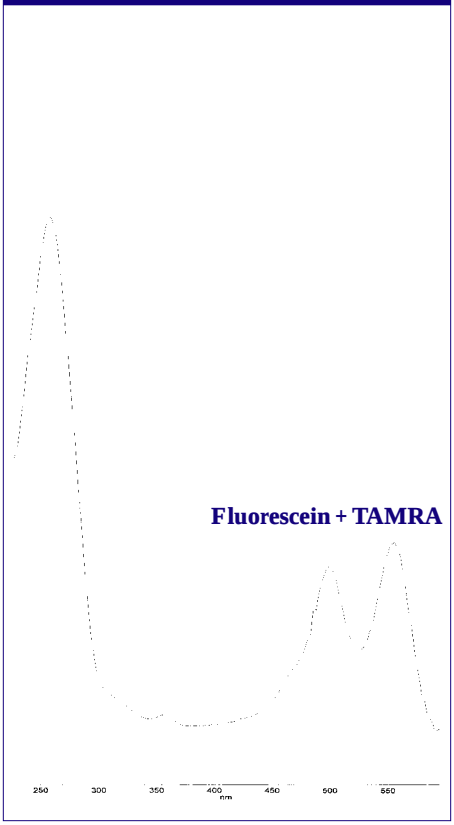


FIGURE 7: RP HPLC OF CRUDE 3'-LABELLED OLIGO

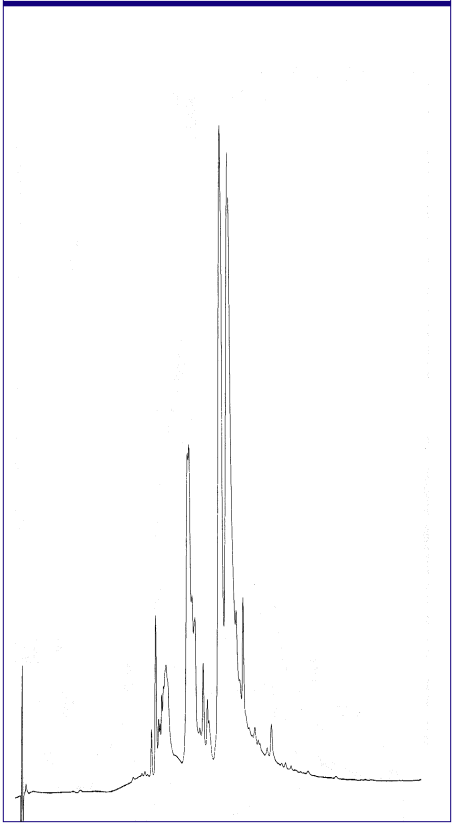


FIGURE 8: RP HPLC OF PURIFIED 3'-LABELLED OLIGO

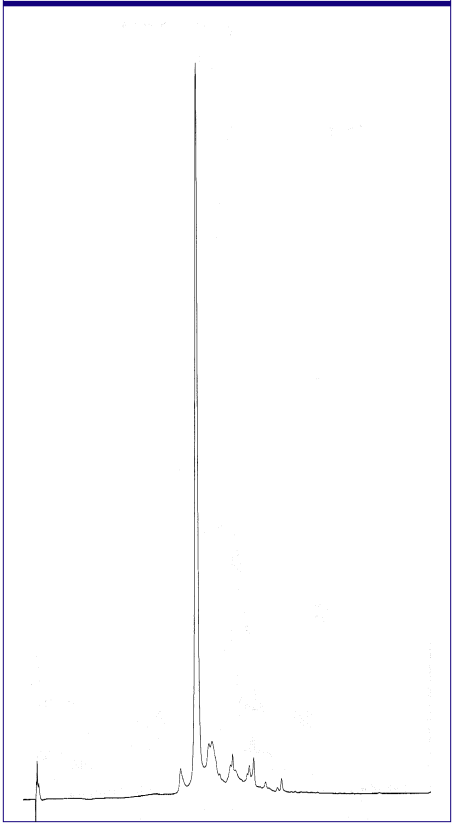
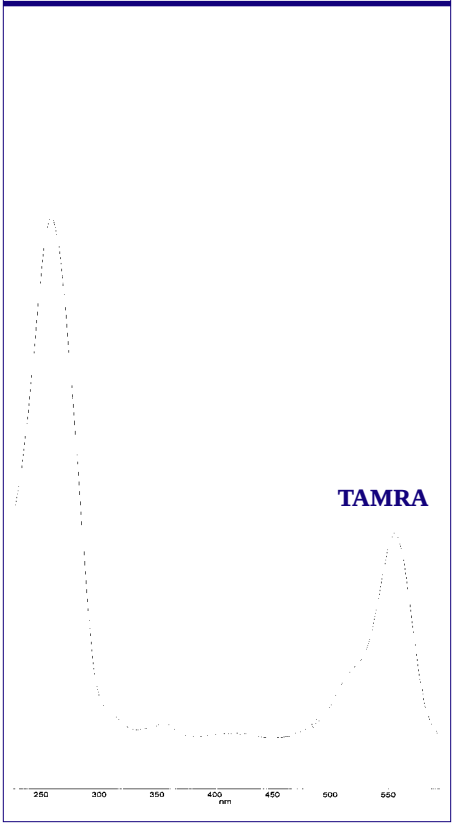


FIGURE 9: UV-VIS SPECTRUM OF PURIFIED 3'-LABELLED OLIGO



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overloading the cartridge results in lower probe yield and purity. Other dye combinations may require more stringent wash conditions depending upon the hydrophobicity of the dyes. Tetramethylrhodamine (TAMRA) dyes are sensitive to ammonia and will degrade in its presence; use UltraMILD DNA monomers (see 'UltraMild DNA Synthesis' in the Glen Research catalog) and deprotect in 50 mM K_2CO_3 in anhydrous methanol for 4 hours at room temperature. Using 0.02 M I_2 for phosphite oxidation will lead to a cleaner probe.

3'-Dye-labelled Oligonucleotides

This protocol has been optimized for 20mers labeled with either TAMRA or fluorescein on the 3' terminus. The loading used was 50-60 OD per Poly-Pak cartridge (approximately 0.5 µmole oligonucleotide). For longer oligos, or oligos that are labelled with a more hydrophobic dye, *e.g.*, Dabcyl, use a higher ratio of ACN to 2% TFA in Solution A.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
3'-Dabcyl CPG 1000	20-5912-01	0.1g	120.00
	20-5912-10	1.0g	975.00
1 µmole columns	20-5912-41	Pack of 4	200.00
0.2 µmole columns	20-5912-42	Pack of 4	120.00
3'-TAMRA CPG 500	20-5910-01	0.1g	120.00
	20-5910-10	1.0g	995.00
1 µmole columns	20-5910-41	Pack of 4	200.00
0.2 µmole columns	20-5910-42	Pack of 4	120.00
5'-Fluorescein Phosphoramidite (6-FAM)	10-5901-95	50 µmole	165.00
	10-5901-90	100 µmole	325.00
	10-5901-02	0.25g	875.00
Poly-Pak™ II Cartridge	60-3100-01	each	12.00
	60-3100-10	Pack of 10	120.00
Poly-Pak™ II Barrel	60-4100-30	Pack of 30	300.00
2.0M Triethylamine Acetate (TEAA) HPLC Grade	60-4110-52	200mL	60.00
	60-4110-57	450mL	120.00
	60-4110-60	960mL	200.00
2% Aqueous Trifluoroacetic Acid	60-4040-57	450mL	36.00

MERMADE INSTRUMENTS

The MerMade DNA synthesizer is a 192 well parallel array synthesizer developed at the McDermott Center for Human Growth and Development,an organized research unit of the University of Texas Southwestern Medical Center at Dallas. Assembled instruments may be purchased from BioAutomation in Plano, TX. Their web site can be found at: http://www.BioAutomation.com

Phosphoramidite monomers are packaged in 240mL amber bottles for dissolving at the rate of 1g/20mL and are connected directly to the instrument.

All solvents and reagents are prepared to our exacting specifications to ensure the highest synthesis efficiency and are passed through a 0.2 micron filter during packaging to eliminate particulate contamination.

Tetrazole in activator solutions is typically at a concentration of around 0.475M, which is close to saturation in acetonitrile. In parallel synthesizers, there is a good possibility that tetrazole will crystallize on tips. This situation would lead to termination of flow which is bad in itself but, even worse, the blockage may be temporary. A solution to this problem is to use 4,5-dicyanoimidazole (DCI) as activator. DCI is typically used at a concentration of 0.25M in acetonitrile which is far below its saturation level at greater than 1.1M.

Regular 500Å supports may be used to fill the wells. However, this requires each plate to be prepared with each nucleoside accurately in all wells. A universal support clearly removes the need for four specific supports and makes preparing plates straightforward. Our universal support has been used in this type of synthesizer. The conditions to eliminate the terminal phosphodiester linkage entirely to 3'-OH are very forcing (e.g., ammonium hydroxide at 80°C for 8 hours minimum). However, the use of ammonium hydroxide for 6 hours at 55°C gives around 50% 3'-OH and oligos prepared this way are perfectly usable sequencing primers. For more stringent applications, the use of 0.4M sodium hydroxide in aqueous methanol gives 100% 3'-OH in 0.5 hours at 80°C. The universal Q support allows cleavage of the oligos from the plate in 2 minutes.

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Monomers			
dA-CE Phosphoramidite	10-1000-5S	5.0g	250.00
	10-1000-1S	10.0g	500.00
dC-CE Phosphoramidite	10-1010-5S	5.0g	250.00
	10-1010-1S	10.0g	500.00
Ac-dC-CE Phosphoramidite	10-1015-5S	5.0g	250.00
	10-1015-1S	10.0g	500.00
dG-CE Phosphoramidite	10-1020-5S	5.0g	250.00
	10-1020-1S	10.0g	500.00
dmf-dG-CE Phosphoramidite	10-1029-5S	5.0g	250.00
	10-1029-1S	10.0g	500.00
dT-CE Phosphoramidite	10-1030-5S	5.0g	250.00
	10-1030-1S	10.0g	500.00
Solvents/Reagents			
Activator			
0.45M Tetrazole, crystalline in Acetonitrile	30-3110-57	450mL	150.00
0.25M DCI in Acetonitrile	30-3150-57	450mL	250.00
Diluent			
Acetonitrile, anhydrous	40-4050-50	100mL	16.00
Cap Mix A			
THF/Lutidine/Ac_2O	40-4010-57	450mL	72.00
Cap Mix B			
16% MeIm in THF	40-4220-57	450mL	96.00
Oxidizing Solution			
0.1M I_2 in THF/Pyridine/H_2O	40-4230-57	450mL	72.00
0.02M I_2 in THF/Pyridine/H_2O	40-4330-57	450mL	72.00
Deblocking Mix			
3% TCA/DCM	40-4140-57	450mL	36.00
Supports			
dA-CPG 500	20-2000-10	1.0g	75.00
dC-CPG 500	20-2010-10	1.0g	75.00
Ac-dC-CPG 500	20-2015-10	1.0g	75.00
dG-CPG 500	20-2020-10	1.0g	75.00
dmf-dG-CPG 500	20-2029-10	1.0g	75.00
dT-CPG 500	20-2030-10	1.0g	75.00
Universal Support 500	20-5000-10	1.0g	95.00
Universal Q Support 500	21-5000-10	1.0g	95.00

MORE NOVEL MONOMERS: NUCLEOSIDE α-THIOTRIPHOSPHATES, 5-HYDROXY-

α-*Thiotriphosphates*

We continue to support the exciting novel technique of Nucleotide Analog Interference Mapping (NAIM). How else can you achieve RNA structural analysis with the resolution of X-ray crystallography with the simplicity and speed of a sequencing gel? Check out a couple of recent reviews[1,2] of this method and be amazed at the power of the technique. We currently maintain a full set of A analogs as α-thiotriphosphates, the "monomers" used in NAIM. (See Page 52 of our 1999 catalog).

However, there are many other uses for α-thiotriphosphates so we have prepared, and will maintain, supplies of regular nucleoside and 2'-deoxy-nucleoside α-thiotriphosphates, as shown in Figure 1.

5-Hydroxymethyl-2'-deoxyUridine

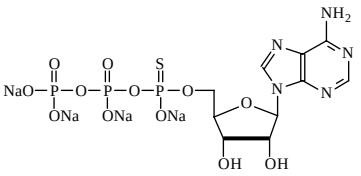
5-Hydroxymethyl-2'-deoxyUridine (HmdU) is formed from Thymidine by the action of peroxide radicals and ionizing radiation. This lesion may cause genetic mutations and chromosomal instability possibly by forming aberrant base pairs with G residues while maintaining its ability in a manner analogous to T to hydrogen bond with A. The cellular repair mechanism consists of excision of the mutant base by hydroxymethyluracil glycosylase to form apyrimidinic sites that are then repaired by excision repair. In our efforts to add to products to aid in studying DNA damage and repair, as well as mutagenesis, we are happy to add this monomer to our growing list of oxidatively damaged nucleosides.

3'- and 5'-Dabcyl Group

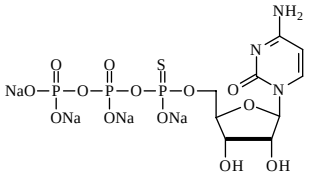
The dabcyl moiety is fairly unique in its ability to quench the fluorescence of virtually any fluorescent tag in its immediate vicinity, making it the preferred quencher for molecular beacon probes. It should be noted that dabcyl quenching does not usually occur by a fluorescence resonance energy transfer (FRET) mechanism. Rather, the

FIGURE 1: STRUCTURES OF α-THIOTRIPHOSPHATES

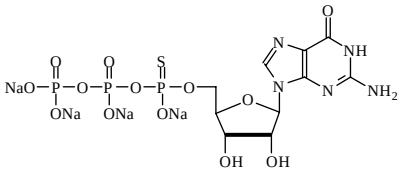
Nucleoside α-thiotriphosphates



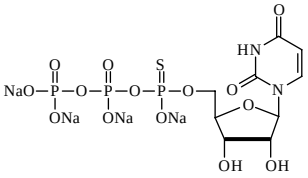
Adenosine



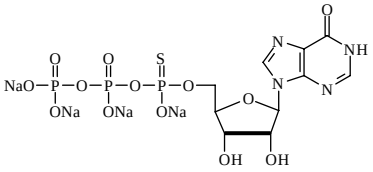
Cytidine



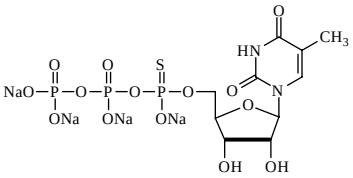
Guanosine



Uridine

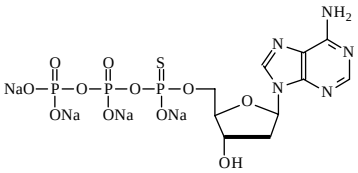


Inosine

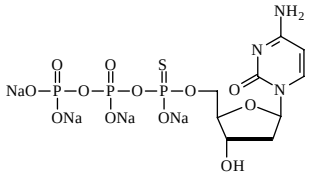


5-Methyl Uridine

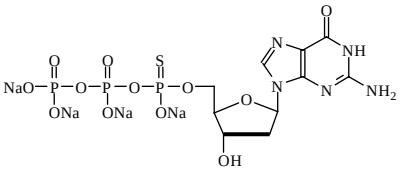
2'-deoxyNucleoside α-thiotriphosphates



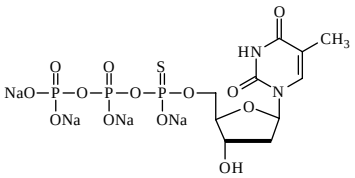
2'-deoxyAdenosine



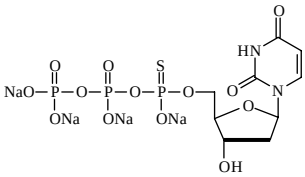
2'-deoxyCytidine



2'-deoxyGuanosine



Thymidine



2'-deoxyUridine

oligonucleotide probe is designed to be self-complementary so that the dabcyl group is in the vicinity of the fluorophore, providing quenching of signal until the probe finds its target. Only then does the probe become linear and, as the dabcyl group is separated from the fluorophore, quenching ceases and fluorescence is observed.

Typically, the dabcyl group is placed at the 3'-terminus and, for that setup, we offer 3'-dabcyl CPG 1000. This support will allow probes up to 60mer in length to be readily prepared. Since the dabcyl group is at least as hydrophobic as any fluorophore, we also offer 5'-dabcyl phosphoramidite. With the most hydrophobic group at the 5'-terminus, it is easier to purify the doubly-labelled oligonucleotide by reverse phase cartridge or HPLC. For example, a probe prepared with fluorescein at the 3'-terminus and dabcyl at the 5'-terminus is considerably easier to purify than the opposite setup.

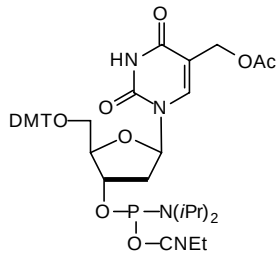
A Novel Fluorescent Nucleotide

Fluorescence Resonance Energy Transfer (FRET) is a powerful tool for elucidating DNA structure and dynamics by probing both the distance and orientation between donor and acceptor fluorophores. However, this yields information regarding DNA structure only indirectly. An interesting alternative would be to excite the bases within the DNA strand itself and observe the energy transfer to a fluorophore. Our most recent fluorescent nucleotide analog, Furano-dT, allows one to do just that. Upon unwinding of a hybridized strand, a five-fold increase in fluorescence is observed at 470 nm when exciting at 260 nm. However, when exciting at 350 nm, no hybridization dependence is seen, which should allow the determination of relative population states. Also, because this fluorophore has only two additional carbon atoms, it may be well tolerated by DNA binding proteins.

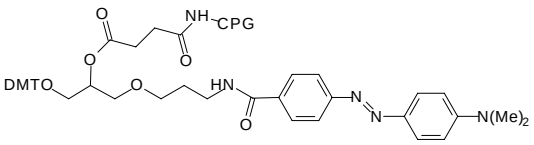
References:

1. S.P. Ryder and S.A. Strobel, *Methods*, 1999, **18**, 38-50.
2. S.A. Strobel, *Curr Opin Struct Biol*, 1999, **9**, 346-52.

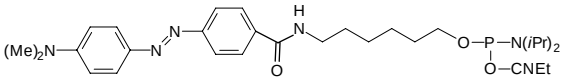
FIGURE 2: STRUCTURES OF NEW PRODUCTS



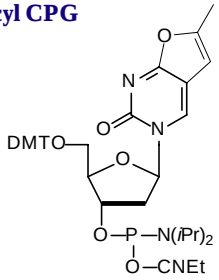
5-Hydroxymethyl-dU



Dabcyl CPG



5'-Dabcyl Phosphoramidite



Furano-dT

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Cytidine α-thiotriphosphate (1.0mM)	80-3010-01	100 µL	75.00
Guanosine α-thiotriphosphate (0.5mM)	80-3020-01	100 µL	75.00
Uridine α-thiotriphosphate (0.5mM)	80-3040-01	100 µL	75.00
5-Methyl-Uridine α-thiotriphosphate (10mM)	80-3093-01	100 µL	75.00
Inosine α-thiotriphosphate (4mM)	80-3050-01	100 µL	75.00
2'-deoxyCytidine α-thiotriphosphate (15mM)	80-1010-01	100 µL	75.00
2'-deoxyGuanosine α-thiotriphosphate (5mM)	80-1020-01	100 µL	75.00
Thymidine α-thiotriphosphate (5mM)	80-1030-01	100 µL	75.00
2'-deoxyUridine α-thiotriphosphate (5mM)	80-1040-01	100 µL	75.00

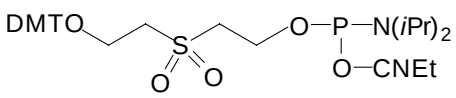
α-Thiotriphosphates are sodium salts in TE buffer, pH7, 10X concentrates. The concentrations shown are optimal for incorporation during polymerase reactions.

5-Hydroxymethyl-dU Phosphoramidite	10-1093-90	100 µmole	225.00
	10-1093-02	0.25g	675.00
3'-Dabcyl CPG 1000	20-5912-01	0.1g	120.00
	20-5912-10	1.0g	975.00
1 µmole columns	20-5912-41	Pack of 4	200.00
0.2 µmole columns	20-5912-42	Pack of 4	120.00
10 µmole column	20-5912-13	Pack of 1	350.00
15 µmole column	20-5912-14	Pack of 1	500.00
5'-Dabcyl Phosphoramidite	10-5912-95	50 µmole	125.00
	10-5912-90	100 µmole	225.00
	10-5912-02	0.25g	650.00
Furano-dT Phosphoramidite	10-1094-90	100 µmole	165.00
	10-1094-02	0.25g	495.00

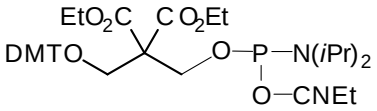
PRODUCT UPDATE - WHICH CHEMICAL PHOSPHORYLATION REAGENT?

So why do we need to be offering two chemical phosphorylation reagents? The original chemical phosphorylation reagent (CPR) (10-1900) works wonderfully giving the 5'-phosphate in virtually quantitative yield. Unfortunately though, it is not compatible with DMT-on purification techniques since the sulfonylethyl group is eliminated in ammonium hydroxide regardless of the presence of the DMT group. Enter CPR II. Coupling efficiency with CPR II (10-1901) is exactly the same as with the original version. However, now you have a choice. Remove the DMT group on the synthesizer and the ammonium hydroxide step generates the 5'-phosphate. Or now you can leave the DMT on and simply purify the oligo using a reverse phase cartridge or HPLC. We are happy to report that CPR II has been awarded US Patent No. 5,959,090 which issued on September 28, 1999.

FIGURE 1: STRUCTURES OF CHEMICAL PHOSPHORYLATION REAGENTS



(1) CPR 10-1900



(2) CPR II 10-1901

ORDERING INFORMATION

Item	Catalog No.	Pack	Price($)
Chemical Phosphorylation Reagent	10-1900-90	100 µmole	50.00
	10-1900-02	0.25g	160.00
Chemical Phosphorylation Reagent II (CPR II)	10-1901-90	100 µmole	60.00
	10-1901-02	0.25g	200.00

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